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Female Carriers of Duchenne Muscular Dystrophy

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Dystrophinopathy, caused by mutations in the *DMD* gene, presents with variable clinical phenotypes ranging from the severe Duchenne muscular dystrophy (DMD) to the milder Becker muscular dystrophy(BMD) forms. DMD is a recessive X-linked form of muscular dystrophy. Two-thirds of mothers of affected males are thought to be DMD carriers. Approximately 2.5-7.8% of female DMD carriers have muscle weakness and are categorized as manifesting DMD carriers. The symptoms of female carriers of DMD range from mild muscle weakness to severe gait problems. The most commonly presented symptom is mild proximal muscle weakness, which is often asymmetric and progressive, but shows variable clinical spectrum with BMD of more severe DMD-like phenotype. Atypical presentations in manifesting carriers are myalgia or cramps without limb weakness, isolated cardiomyopathy and camptocormia. Multiplex PCR and MLPA analysis are common techniques to identify mutations in the *DMD* gene. Relationship between X-chromosome inactivation and clinical severity is not clear. Female carriers of DMD are not less common, and they have an important role of birth of a male DMD.

Key words: Dystrophinopathy, Duchenne muscular dystrophy, Female carrier, Multiplex ligation-dependent probe amplification

Introduction

Duchenne muscular dystrophy (DMD) is a recessive X-linked form of muscular dystrophy. The milder form of this disease is called Becker muscular dystrophy(BMD).[1] The disorder is also called dystrophinopathy because it caused by mutations in the *DMD* gene, which encodes dystrophin, on Xp21.[2] Most heterozygous female carriers of DMD are subclinical. Therefore, identification of dystrophinopathy carriers may be only considered on clinical grounds such as clear X-linked family history of muscular dystrophy. However, myopathic muscle biopsy and advanced molecular diagnostic analysis can be used to identify the carrier state of DMD without a positive family history of dystrophinopathy , which is present in 10% of women with hyperCKemia.[3] In addition, two-thirds of mothers of affected males are thought to be DMD carriers. Approximately 2.5-7.8% of female DMD carriers have muscle weakness and are categorized as manifesting DMD carriers.[1, 2, 4] Therefore, here we reviewed recent studies to meet the need for a better understanding about the characterization of female carriers of DMD.

What is dystrophinopathies?

Duchenne's description of DMD was published in 1861. He referred to the disease as "hypertrophic paraplegia of infancy due to a cerebral cause".[5] However, in 1868, he performed muscle biopsies and recognized that it was of muscular origin. He proposed the criteria for diagnosing this disease and reported the results of muscle biopsy which showed replacement of muscle by fat and connective tissue.[6] Gowers, in 1886, noted that cases

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always occurred on the mother's side of the family.[7] Depending on the advancement of molecular genetics, mapping of the gene responsible for DMD was available to band p21 of the short arm of the X chromosome (Xp21).[8-11] Subsequently, Kunkel's group identified the muscle protein dystrophin which is encoded by the *DMD* gene.[12] The full-size dystrophin is 427 kDa in molecular mass[12] and its distribution is almost the same in both slow and fast fiber types.[13] In addition, the extent of dystrophin expression is associated with clinical severity of dystrophinopathy patients.[13] Dystrophin, located in the sarcolemma of muscle fibers, is almost absent in DMD and decreased in BMD. These advances were able to make diagnosis and genetic counseling more accurate.

Dystrophinopathies in female patients

Random inactivation of one of the two X chromosomes occurs during early embryonic development, leaving active 50% of the maternally derived chromosomes and 50% of the paternally derived ones.[14] Non-random X-chromosome inactivation induce fewer than 50% of the nuclei which may have the normal dystrophin gene, resulting in clinical manifesting female carriers.[15] Some reports described cases of X-autosomal translocations with breakpoints at Xp21 resulting in preferential inactivation of the normal chromosome.[8, 11] If the X chromosome of turner syndrome patient (karyotype XO) carries a mutated dystrophin gene, her daughter can be symptomatic.

Clinical presentation of female carrier of DMD

DMD is the most common form of childhood muscular dystrophy, with an incidence of approximately 1 in 3,600-6,000 live male births and a prevalence of approximately 3 per 100,000.[16, 17] The symptoms of DMD female carriers range from mild muscle weakness to severe gait problems. Although manifesting girls usually show much milder symptoms than boys, a few cases have disease severity similar to that seen in affected boys.[3, 18, 19] The most commonly presented symptom is mild proximal muscle weakness, which is often asymmetric and progressive. There are variable clinical patterns from BMD-like to severe DMD-like phenotype.[20] The prevalence of manifesting female carriers of DMD is slightly different in each study. Recent study reported 22% of female carriers manifested the symptoms, with 19% demonstrating muscle weakness measured by manual muscle testing and 8% presenting with dilated cardiomyopathy.[21] A similar prevalence of females with symptoms was observed by other group, with 12% presenting with muscle weakness and 7% displaying cardiomyopathies.[22] Some manifesting carriers only present myalgia or cramps without limb weakness.[23] In addition, unusual cases such as isolated cardiomyopathy without skeletal muscle weakness[24-26] and developmental delay in childhood have been reported.[23] Also, there have been reports of manifesting carriers of DMD who initially presented with camptocormia which is forward flexion of the thoracolumbar spine that abates in the supine position.[27]

Imaging evaluation of female carrier of DMD

Serum creatine kinase levels are commonly elevated in manifesting carriers of DMD.[28] Magnetic resonance imaging (MRI) is performed for more comprehensive assessment of muscles. There was a report about elevation of reported proton spin lattice relaxation time (T_1) in female carriers elevated in the gluteals, vastus lateralis, and gastrocnemius compared with the control group and correlation with the distribution of muscle edema.[29] Elevated spin-spin or tranverse relaxation time (T_2) in DMD carriers was also observed following muscle damage and inflammation/edema.[30, 31] Quantitative MRI measures were used to evaluate muscle composition.[32] In female carriers of DMD, there was considerable heterogeneity among lipid fraction measures compared with the control groups during 21 months of a longitudinal study.[32] Although specific findings of imaging evaluation in female carriers of DMD has not yet been established, depending on the advance of neuroradiology, these invasive techniques could be strong tools for evaluation of carrier status.

Insights of diagnostic strategies of female carrier of DMD: diagnosis, novel pathological and genetic evidence

1. Pathology

Muscle biopsy is used for diagnosis in cases of clinically suspicious of dystrophinopathy. It can provide information about the amount and molecular size if the protein is present. Muscle biopsy is usually not necessary when genetic testing is positive. However, pathological evidence is helpful for patients with a family history of DMD, but no family mutation is known.[18]

Dystrophin labeling of muscle biopsies from manifesting carriers

shows a mosaic pattern, with reduced or absent dystrophin.[33, 34] Immunohistochemistry findings are concordant with western blot analysis for dystrophin.[34] No association is seen between the pattern of distribution of dystrophin and clinical severity of manifesting carriers.[35] However, a few cases revealed that the absence of dystrophin expression and unstained fiber presumably affected the more severe phenotype.[20] Moreover, dystrophin expression can vary in different muscle groups of DMD carriers.[36]

2. Genetic test

The dystrophin gene have majority of deletion mutation, approximately 61.9-73% in western, northerm and Asian populations.[37-39] The frequency of deletions of the DMD gene is greater in affected males resulting from a female gametic mutation (75%) than in those resulting from a male genetic mutation (56%).[40] *DMD* gene partial duplications account for up to 6% of DMD and BMD cases.[41]

Dystrophin mutation is able to be detected by Multiplex PCR[42] and multiplex ligation-dependent probe amplification (MLPA).[43]

Multiplex PCR is a common technique to identify mutations of the *DMD* gene and can detect approximately 98% of deletions.[34] Since multiplex PCR is the least expensive and sensitive method, it is the first method of choice by clinicians when the patient is highly suspicious of DMD.[41] Recently, several reports showed that MLPA is a more accurate method for mutation analysis of DMD than multiplex PCR. MLPA can detect the presence of deletions, duplication and abnormal DNA methylation. In particular, MLPA is the gold standard for molecular analysis of large number of genes.[44] Therefore, if the patient is clinically suspected of DMD or is a female carrier of DMD but shows negative results from multiplex PCR, MLPA is recommend for mutation analysis.[45] MLPA probes are sensitive to small changes within the sequence detected by the probe. A single nucleotide change very close to the probe ligation site can result in a decreased probe signal, thus mimicking a deletion.[46] Therefore, it is strongly recommended to confirm all MLPA findings with another method, especially in cases of single probe deletions.[46, 47]

Furthermore, dystrophin gene sequencing is used to analyze single-condition amplification/internal primers and multiplex amplifiable probe hybridization for detecting point mutations or small deletions/insertions if deletion/ duplication testing is negative.[48, 49]

Full characterisation of the mutation (deletion endpoints or exact position of any point mutation) is helpful to investigate the clinical variability of dystrophinopathy[50, 51] and recent trials about mutation-specific treatment.[52-54]

3. X-chromosome inactivation (XCI)

Symptomatic carriers of DMD without chromosomal rearrangements have been explained by skewed X-chromosome inactivation related to preferential expression of the mutant allele.[36, 55, 56] The nonrandom X-inactivation pattern of manifesting carriers of DMD showed derivative X remaining active as a saving mechanism against disease-expression.[11, 57] Therefore, the extent of X-chromosome inactivation skewing is correlated with clinical phenotype.[58] Nevertheless, some studies reported no significant association between X-chromosome inactivation pattern and clinical severity.[59] Prognostic value of X-chromosome inactivation is also controversial.[58, 60, 61]

Conclusion

Female carriers of DMD are not less common, and they have an important role in male DMD. Comprehension of characterization of female carriers of DMD is helpful for establishment of diagnostic approaches in patients who may have in the past been diagnosed as having autosomal recessive limb-girdle muscular dystrophy or unknown myopathy with a negative family history.

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